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Biologicheskii metod diagnostiki sibirskoi yazvy

(Biological method of diagnosis of anthrax)

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(In Russian)

It is necessary to conduct bacteriological research (YA. E. Shostak, 1924; N. I. Rozenov, 1952; M. V. Revo, 1958; G. P. Rudnev, 1959) in order to diagnose anthrax in animals and man; one of the component parts of this research is the setting up of a biological test on laboratory animals. Whereupon the death of animals from anthrax occurs at different times (from 1 to 10 days).

We did not find in literature any description of an accelerated method of diagnosis of anthrax, which would be confirmed by a biological test. Aiming at solving this problem, we utilized animals with a reduced resistance; this was already practiced in the diagnosis of various infectious diseases (E. V. Vorenina, 1942; S. L. Biyakher, 1958; K. S. Karpuzidi, M. S. Droxhevkine, T. I. Kharitonova, D. I. Kolotienko and Z. D. Khakhina, 1961).

At the present time cortisone is used for the reduction of resistance in animals (M. P. Pokrovskaya, S. L. Blyakher, A. N. Meshalov, 1958; I. Franek, 1959), irradiation with x-rays (N. A. Izraitel', A. I. Krasil'nikov, 1958; M. S. Drozhevkina, T. I. Kharitonova, D. I. Kolotienko and K. S. Karpuzidi) and narcosis (I. MU. Uchitel', 1951; Kh. Ayurzan, 1956).

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In our experiments we utilized the ether narcosis. In a preliminary test we studied the dynamics of distribution of the pathogen of anthrax in the organism of guinea pigs in a subcutaneous administration to them of 0.2 ml of the twenty-four-hour-old bouillon culture of Bac. anthracis, diluted 1:10.

At different times after the introduction of the culture, the animals were killed, the material from them was examined bacteriologically and bacterioscopically.

This experiment has shown that after the infection of guinea pigs in the smears from the place of infection anthrax bacilli, with the characteristic capsules, were not discovered earlier than after 15 hours, while the growth of culture appears in 9 hours and only in seedings from the place of injection.

In order to find an accelerated method of diagnosis of anthrax, we set up experiments on 24 guinea pigs, weighing 370-570 g; 13 among these were subjected to a twofold ether anesthesis according to the following method. One or two guinea pigs were placed into a jar, on the bottom of which we put cotton, saturated with anesthetic ether (3-5 ml) and covered it with a lid. As soon as the animals fell asleep we stopped the giving of ether; we transferred the guinea pigs to another vessel (for 15-20 minutes); after this we infected them subcutaneously with a twenty-four-hour-old bouillon culture of Bac. anthracis in a dose of 0.02 ml. So as to reduce the resistance of the organism of the guinea pigs still more (Begin p. 59) in 3-5 minutes after infection we again subjected them to narcosis. The session continued 7-10 minutes.

The control (11) guinea pigs were infected with the same culture but without the narcosis. In three-six-nine hours after the infection the

experimental and the control guinea pigs were killed, dissected and smears and seedings were made from the spleen, liver, lungs, kidneys, blood, regional lymph nodes, as well as from the place of injection (table 1).

It is seen from table 1, that we succeeded isolating a culture of Bac. anthracis from the killed experimental guinea pigs from the place of infection already in three hours after infection, and, in six hours, in the smears we discovered capsular forms of this microbe in large numbers, and, at the same time, from the seedings from the place of injection and from the regional lymph nodes we obtained a culture of the pathogen of anthrax; while from the control guinea pigs the pure culture of the anthrax bacillus was isolated from the place of infection in nine hours (in one guinea pig). But the microscopic examination did not produce any positive results in one single case.

On the basis of results of this experiment we came to conclusion that the ether anesthesia is a convenient and reliable method for the setting up of the accelerated biological test for anthrax.

Identification is needed in the biological examination of the material for anthrax of the isolated (Begin p. 60) culture with the morphologically similar saprophytes. Therefore, for the purpose of finding out if the weakening of the organism of experimental animals by narcosis does not lead to manifestation in it of pathogenic characteristics of microbes from the group of anthracoids, we set up an experiment with 76 guinea pigs. Among them we infected 36 with the culture of Bac. anthracis, 24 -with Bac. pseudoanthracis and 16 - Bac. anthracoides. Before the infection and after it, 38 animals were subjected to the ether anesthesia according to the above described method (table 2). (Table 2 is on a separate page.)

Table I

Data of the microsconic and bacteriological researches of the material from guinea pigs, infected with anthrax after a double reaction of the anesthetic ether.

Time of examination of a guinea pig after infection in:	Results of examination													
	from the place of infection		lymph node		spleen		liver		lung		blood		kidney	
	microscopy	seeding	microscopy	seeding	microscopy	seeding	microscopy	seeding	microscopy	seeding	microscopy	seeding	microscopy	seeding
Experimental														
3 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4 "	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5 hours	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5 "	-	+	-	-	-	-	-	-	-	-	-	-	-	-
6 "	+	+	-	+	-	-	-	-	-	-	-	-	-	-
6 "	+	+	-	+	-	-	-	-	-	-	-	-	-	-
6 "	+	+	-	+	-	-	-	-	-	-	-	-	-	-
6 "	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6 "	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9 "	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9 "	+	+	-	+	-	-	-	-	-	-	-	-	-	-
9 "	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Died (in 58 hrs)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Central														
3 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9 "	-	+	-	-	-	-	-	-	-	-	-	-	-	-
9 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9 "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Died (in 58 hrs)	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Footnote: - negative results - + positive.

It is seen from table 2 that in the group of guinea pigs, infected with culture Bac. anthracis, the same results were obtained as in the preceding experiment. In the groups of animals, infected with cultures of Bac. pseudoanthracis and Bac. anthracoides, the results coincided: all the experimental and the control animals remained alive. The microscopic examination of smears produced negative results in all cases. We succeeded in isolating the administered microbe in three hours after infection in seedings from the places of infection, while in nine hours it already could not be isolated.

Thus, in guinea pigs, which underwent the ether anesthesia, the saprophytes do not produce any formation of capsular forms of microbes at the place of infection and do not cause the death of animals.

In subsequent experiments we examined guinea pigs, which underwent ether anesthesia, for the accelerated diagnosis of anthrax in utilizing for injection pathological material from a dead animal (spleen and edematous liquid from the place of injection). In six hours after infection of guinea pigs, we discovered in smears, made from the place of infection, capsular (Begin p. 61) bacilli characteristic to the anthrax pathogen.

It was established, in the special experiment on 24 guinea pigs, that in the infection of animals with the spore culture of Bac. anthracis (dose 500 and 52,000 spores) in the smears from the place of infection from all the animals, treated with ether, one succeeds in obtaining the capsular form of the anthrax bacillus in 12 hours and obtain the microbe culture in 6 hours after infection.

In the control guinea pigs positive results were obtained by microscopy only from the dead animals.

Table 2

Results of microscopic and bacteriological examinations of guinea pigs, infected with Bac. anthracis, Bac. pseudoanthracis, and Bac. anthracoides.

Infected with the microbe culture	Amount of guinea pigs in the experiment	Time of research after infection: in:	Indices of the research													
			from the place of infection		lymph node		spleen		liver		lung		blood		kidneys	
			microscopy positive	growth of culture	microscopy	growth of culture	microscopy	growth of culture	microscopy	growth of culture	microscopy	growth of culture	microscopy	growth of culture	microscopy	
Bac. anthracis (with narcosis)	6	3 hours	2	6	-	2	-	2	-	-	-	-	-	-	-	-
	6	6 hours	6	6	-	2	-	1	-	-	-	-	-	-	-	-
	3	9 hours	3	3	-	3	-	1	-	-	-	-	-	-	-	-
Died in 44 hours	3	3 hours	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Bac. anthracis (without narcosis)	6	3 hours	1	3	-	-	-	-	-	-	-	-	-	-	-	-
	6	6 hours	1	3	-	1	-	-	-	-	-	-	-	-	-	-
	3	9 hours	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Died in 44 hours	3	3 hours	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Bac. pseudoanthracis (with narcosis)	3	3 hours	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	6 hours	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	3	9 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Were killed in 11 days	3	---	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bac. pseudoanthracis (without narcosis)	3	3 hours	-	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	6 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	9 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Were killed in 11 days	3	---	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bac. anthracoides (with narcosis)	2	3 hours	-	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	6 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	9 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Were killed in 11 days	2	---	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bac. anthracoides (without narcosis)	2	3 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	6 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	9 hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Were killed in 11 days	2	---	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Footnote: [illegible] denote the number of positive results, while the sign - denotes the [illegible] results.

Experiments with the utilization of a single ether anesthesia (before or after the infection) did not produce any results; apparently, a single utilization of anesthesia reduces the resistance of the animal only slightly and, therefore, does not lead to the acceleration of the diagnosis.

Later on we utilized the method of seeding the specimen obtained by puncture from the place of infection. This method of research is simple and convenient. Having infected one guinea pig, it can be subjected to research at various times.

Technique of research. In guinea pigs hair is removed from the place earmarked for injection; it is rubbed with alchol and the material under examination is then administered. After this, at different times, material is removed with a sterile syringe from the place of injection and a smear is made. The liquid, remaining in the syringe, is seeded on meat-peptone agar. The smear is fixed in the Nikiforov's mixture for 15-30 minutes, and stained by one of the methods which reveals the capsule.

During our experiments, in the examination of the specimen obtained by puncture, in 3 hours after infection in guinea pigs, which underwent ether anesthesia, we discovered capsular forms of Bac. anthracis (1-2 chains in the field of vision) in the smears, while in the seeding we obtained a pure culture of anthrax bacilli.

In taking a specimen by puncture in 6, 9, and 12 hours we discovered in the smears a large amount of the capsular form of the microbe and in the seedings we isolated pure culture of the anthrax pathogen.

Conclusions

1. In order to speed up the diagnosis of anthrax for the biological test, it is expedient to utilize guinea pigs, which underwent a double reaction of the ether anesthesia; at such a time the period of research is reduced to three-six hours.

2. Bacteriological diagnosis of anthrax can be speeded up by means of examin ation of the specimen obtained through puncture from the place of secretion of suspicious material, or of a regional lymph node of the guinea pig, utilized as a biological specimen.

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